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Application Number

08/376,327

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703-517-9458

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US006200806B1

31

(12) **United States Patent**  
Thomson

(10) Patent No.: **US 6,200,806 B1**  
(45) Date of Patent: **Mar. 13, 2001**

(54) **PRIMATE EMBRYONIC STEM CELLS**

(75) Inventor: **James A. Thomson, Madison, WI (US)**

(73) Assignee: **Wisconsin Alumni Research Foundation, Madison, WI (US)**

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/106,390**

(22) Filed: **Jun. 26, 1998**

**Related U.S. Application Data**

(60) Division of application No. 08/591,246, filed on Jan. 18, 1996, now Pat. No. 5,843,780, and a continuation-in-part of application No. 08/376,327, filed on Jan. 20, 1995, now abandoned.

(51) Int. Cl.<sup>7</sup> ..... **C12N 5/08; C12N 5/06**

(52) U.S. Cl. .... **435/366; 435/325**

(58) Field of Search ..... **800/8; 435/325, 435/366**

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(57) **ABSTRACT**

A purified preparation of primate embryonic stem cells is disclosed. This preparation is characterized by the following cell surface markers: SSEA-1 (-); SSEA-4 (+); TRA-1-60 (+); TRA-1-81 (+); and alkaline phosphatase (+). In a particularly advantageous embodiment, the cells of the preparation are human embryonic stem cells, have normal karyotypes, and continue to proliferate in an undifferentiated state after continuous culture for eleven months. The embryonic stem cell lines also retain the ability, throughout the culture, to form trophoblast and to differentiate into all tissues derived from all three embryonic germ layers (endoderm, mesoderm and ectoderm). A method for isolating a primate embryonic stem cell line is also disclosed.

**11 Claims, 8 Drawing Sheets**